Are muscarinic receptors really voltage sensors?

Malka Cohen-Armon

The Sackler School of Medicine, and Sagol School of Neuroscience, Tel-Aviv University, Tel-Aviv 69978, Israel.

This opinion paper addresses the hypothesis that the muscarinic receptor contains an embedded voltage sensor, which appears in several papers describing this un-expected phenomenon (1-5). According to this assumption, a voltage-induced charge movements accompanied by muscarinic receptor's 'gating currents' underlie the depolarization-induced modulation of the receptor's affinity for agonists (1-3).However, here we show that this un-proved 'voltage sensing' of muscarinic receptors (6) is inconsistent with evidence, indicating that the open configuration of voltage dependent sodium channels (VDSC) is required for the depolarization-induced affinity modulation of muscarinic receptors (7-9).

Different affinities of muscarinic receptors for their cholinergic agonists were identified four decades ago (10). The voltage-induced modulation of muscarinic receptors' affinity for their agonists was first identified in rat brain-cortex, brain-stem and atria (11), and it was further investigated in the M2 muscarinic receptor subtype (3). The voltage-induced modulation of the affinity of muscarinic receptors for different ligands is apparently one of their common features (5).
Furthermore, the role of the open configuration of VDSC gating in the voltage-induced affinity modulation of muscarinic receptors for their agonists has been identified (7-9). Evidence indicating an interaction between muscarinic receptors and VDSC included muscarinic-induced opening of VDSC (9). This was indicated by muscarinic induced TTX sensitive $^{22}Na^{+}$ inwards flux in brain synaptoneurosomes (9), and by the binding of labeled toxins to their sites in the open VDSC (7-9,12-16). The binding of specific toxins to open VDSC either kept the VDSC in their open configuration (e.g., Batrachotoxin (BTX), the S-enantiomer of the cardio-tonic drug DPI (13-16)), or disrupted the open configuration of VDSC (e.g., the R-enantiomer of the cardio-tonic drug, DPI;16). In these experiments, muscarinic cholinergic agonists dose-dependently induced TTX-sensitive $^{22}Na^{+}$ influx in brain synaptoneuro-somes, which was blocked by muscarinic antagonists (9; Figure 1). In accordance, muscarinic agonists dose-dependently enhanced the specific binding of [^{3}H]BTX, even when Na^{+} current was blocked and synaptoneurosomes were not depolarized (9; Figure 1). Thus, according to these results, muscarinic cholinergic agonists induced the opening of VDSC at resting potential in brain synaptoneurosomes.

Notably, a reciprocal effect of VDSC gating on the binding of agonists to muscarinic receptors was identified (7,8). Membrane depolarization extensively decreased the high affinity of muscarinic receptors for [^{3}H]acetylcholine in brain synaptoneurosomes (7, 17). This voltage-induced

change in the high affinity of muscarinic receptors was dependent on the open state of VDSC, while Na^+ current was blocked (7,8). A substantial reduction in the high-affinity binding of [^{3}H]acetylcholine to muscarinic receptors was measured in depolarized brain synaptoneurosomes treated with BTX, or the S-enantiomer of the cardio-tonic drug DPI, both keeping the open configuration of VDSC (7,12-16) (Figure 2). However, no reduction in the high affinity binding of [^{3}H]acetylcholine to muscarinic receptors was measured in depolarized synaptoneurosomes when the open configuration of the VDSC was prevented by the R-enantiomer of the cardio-tonic drug DPI (7,16) (Figure 2). Thus, the open configuration of VDSC (but not Na^+ current) was required for the depolarization-induced change in the muscarinic receptors affinity for their agonist (7).The binding of muscarinic antagonists was not affected by membrane depolarization (7-9).

Several findings attributed the high-to-low affinity modulation of muscarinic receptors for their agonists to a depolarization-induced activation of their coupled G-proteins (ref 6,7, 11,17,18,19). The activation of some types of G-proteins by muscarinic agonists, including the activation of the pertussis toxin sensitive Gi- and Go-proteins, was measured by the exchange of GDP with labeled GTP (17). A depolarization-induced exchange of GDP by GTP was measured in Go-proteins (17). Notably, the open configuration of the VDSC was a pre-requisite for this voltage-dependent activation of Go-proteins in the brain synaptoneurosomes, while Na^+ current was blocked (17). Muscarinic agonists were not required, and muscarinic antagonists did not prevent the depolarization-induced GDP exchange by GTP in Go-proteins (17). Thus, according to these results, the depolarization-induced opening of VDSC was a pre-requisite for both depolarization-induced activation of Go protein, and the depolarization-induced affinity modulation of muscarinic receptors (7, 8,17).
A possible implication of the depolarization-induced activation of G-proteins in the depolarization-induced affinity modulation of the muscarinic receptors was examined (7,17). Modifications preventing the activation of G-proteins did not interfere with the muscarinic induced opening of VDSC (9; Figure1), suggesting no intervention of G-proteins in the effect of muscarinic agonists on VDSC opening.
In contrast, the depolarization-induced modulation of the muscarinic receptors' affinity for agonists was affected by G-protein activation in brain synaptoneurosomes (17). Binding of irreplaceable GDP to G-proteins (GDPβS), or pertussis toxin-catalyzed ADP-ribosylation of the α-subunit of G-proteins, prevented the activation of G-proteins both by muscarinic agonists and by membrane depolarization in brain synaptoneurosomes (17). Furthermore, these modifications in G-proteins also interfered with the effect of membrane depolarization on the muscarinic receptors' affinity for agonists (17). Notably, the VDSC gating was implicated in the effect of these modifications in G-proteins on the affinity of muscarinic receptors (17). A permanent binding of GTP to G-proteins (Gpp(NH)p) reduced the high affinity of muscarinic receptor for their agonists only when the open configuration of the VDSC was preserved (17). Treatments preventing the opening of VDSC prevented the effect of G-protein activation on the high–to-low affinity modulation of muscarinic receptors for [^{3}H]acetylcholine (17) (Figure 3). Thus, according to these results, the open configuration of VDSC was a pre-requisite for the muscarinic receptors' affinity modulation, either induced by membrane depolarization or by the activation of the receptors coupled G-proteins.

In accordance, these findings outline a depolarization-induced modulation of the muscarinic receptors affinity for agonists, which is dependent on depolarization-induced opening of the VDSC and on depolarization-induced activation of muscarinic coupled Go-proteins (7, 8, 17).
These findings are inconsistent with the assumption of a voltage sensor embedded in muscarinic receptors, modifying their affinity in response to membrane depolarization.

Acknowledgement: The Late Prof Haim Garty, Weizmann Institute of Science, made a major contribution to this work.

References

1. Dekel D., Bentulila Z., Tauber, M., Ben-Chaim, Y. G-protein coupled receptors regulated by membrane potential. Int. J. Mol. Sci. 2022,23,13988
2. Barchad-Avitzur O, Priest MF, Dekel N, Bezanilla F, Parnas H, Ben-Chaim Y. A Novel Voltage Sensor in the Orthosteric Binding Site of the M2 Muscarinic Receptor**.**Biophys J. 2016;111:1396-1408
3. Ben-Chaim, Y., Chanda, B., Dascal, N., Bezanilla, F., Parnas, I., Parnas, H. Movement of 'gating charge' is coupled to ligand binding in a G-protein-coupled receptor. Nature 2006,444, 106-109
4. Friedman, S., Tauber, M. & Ben-Chaim, Y. Sodium ions allosterically modulate the M2 muscarinic receptor. *Sci Rep* 2020, 10, 11177
5. Rinne A, Mobarec JC, Mahaut-Smith M, Kolb P, Bünemann M. The mode of agonist binding to a G-protein-coupled receptor switches the effect that voltage changes have on signaling. Sci Signal. 2015;8 (401):110-1118.
6. Mahaut-Smith M.P., Martinez-Pinna, J., Gurung, I.S. A role of membrane potential in regulating GPCR? Cell, 2008, 29, 421-429
7. Cohen-Armon M, Sokolovsky M Evidence for involvement of the voltage-dependent Na^+ channel gating in depolarization-induced activation of G-proteins. J Biol Chem. 1993;268:9824-9838.
8. Cohen-Armon, M., Kloog, Y., Henis, Y.I., Sokolovsky, M. Batrachotoxin changes the properties of the muscarinic receptor in rat brain and heart: possible interaction(s) between muscarinic receptors and sodium channels. Proc Natl Acad Sci U S A. 1985;82:3524-3527.
9. Cohen-Armon M, Garty H, Sokolovsky M.G-protein mediates voltage regulation of agonist binding to muscarinic receptors: effects on receptor-Na^+ channel interaction. Biochemistry. 1988;27:368-374.
10. Birdsall, N.J.M., Hulme, E.C. Muscarinic receptor subclasses. Trends in Pharmacol. Sci. 1983, 4, 459-463

11. Cohen-Armon M, Sokolovsky M Depolarization-induced changes in the muscarinic receptor in rat brain and heart are mediated by pertussis-toxin- sensitive G-proteins..J Biol Chem. 1991;266:2595-605.
12. Yarov-Yarovoya, V., Decaena, P.G., Westenbroeka, R.E., Chien-Yuan, P., Scheuera,T.S., Bakerb, D., Catterall, W.A. Structural basis for gating charge movement in the voltage sensor of a sodium channel Proc.Natl. Acad. Sci. 2012, 109, E-93-E102.
13. Catterall, W.A., Cestele, S., Yarov-Yarovoy, V., Yu, F.H., Konoki, K., Scheuer, T. Voltage-gated ion-channels and gating modifier toxins. Toxicon, 2007, 49,124-141
14. Khodorov B.I., Batrachotoxin as a tool to study voltage-sensitive sodium channels of excitable membranes. Prog. Biophys. Mol. Biol. 1985, 45, 57-148
15. De Lera Ruiz, M., Kraus, R.L., Voltage-gated Sodium Channels: structure, function, pharmacology and clinical indications J.Med. Chem. 2015, 58, 7093-7118
16. Romey, G., Quast, U., Pauron, D., Frelin, C., Renaud, J.F., Lazdunski, M. Na^+ channels as sites of action of the cardioactive agent DPI201-106 with agonist and antagonist enantiomer Proc. Natl. Acad. Sci. 1987, 84, 896-900
17. Anis Y, Nürnberg B, Visochek L, Reiss N, Naor Z, Cohen-Armon M. Activation of Go-proteins by membrane depolarization traced by in-situ photoaffinity labeling of Gαo-proteins with [α^{32}P]GTP-azidoanilide. J Biol Chem. 1999;274:7431-440.
18. Ben-Chaim Y, Broide C, Parnas H._The coupling of the M2 muscarinic recept to G-protein is voltage dependent. PLoS One. 2019;14:e0224367.
19. Jiang, M., Bajpayee, N.S., Molecular mechanisms of Go signaling. Neurosignals 2009, 17, 23-41

Figure legends:

Figure1

Upper frame: TTX-blockable $^{22}Na^+$ uptake (circles) and [^{3}H]BTX binding to pertussis toxin treated synaptoneurosomes (squares) as function of carbamylcholine concentration in the presence (●, ■) and absence (○, □) of atropine (0.1-1 μM).

Lower frame: [^{3}BTX] (0.6 μM) binding to synaptoneurosomes in the presence of carbamylcholine (●, ■), or carbamylcholine plus 0.1 μM atropine (○, □). [^{3}H]BTX binding was measured following treatment with pertussis toxin (circles), or in the presence of 1μM TTX (squares). (9)

Figure 2
Effects of DPI enantiomers on the high affinity specific binding of [^{3}H]Acethylcholine ([^{3}H]AcCh) to muscarinic receptors in rat brain synaptoneurosomes at resting potential (in 4.7 mM [K^+] buffer, ○, △) and upon depolarization (in 50 mM [K^+] buffer, ●, ▲). [^{3}H]AcCh binding measured in untreated synaptoneurosomes (black lines, circles) and in synaptoneurosomes treated (grey lines, triangles) with the S-enantiomer of DPI (5 μM) in the presence of 1 μM TTX (**A**), or with the R-enantiomer of DPI (5 μM) (**B**), are presented. The non-specific binding of [^{3}H]AcCh was measured in the presence of 1 μM atropine. (7)

Figure 3
The effect of Gpp(NH)p (200 μM) on the high-affinity binding of [^{3}H]AcCh to muscarinic receptors measured in rat brain-stem membranes in the presence of the R-enantiomer and the racemic mixture of the cardiotonic drug DPI.
A. [^{3}H]AcCh binding to the muscarinic receptors in the presence (■) and in the absence (□) of Gpp(NH)p.
B. [^{3}H]AcCh binding was measured in membranes treated with the R-enantiomer (grey curves, triangles) and the racemic mixture of the cardiotonic drug DPI (black curves, circles) in the absence (○, △), and in the presence of Gpp(NH)p (●, ▲). The non-specific binding of [^{3}H]AcCh to the muscarinic receptors was measured in the presence of 1 μM atropine. (17)

Figure 1

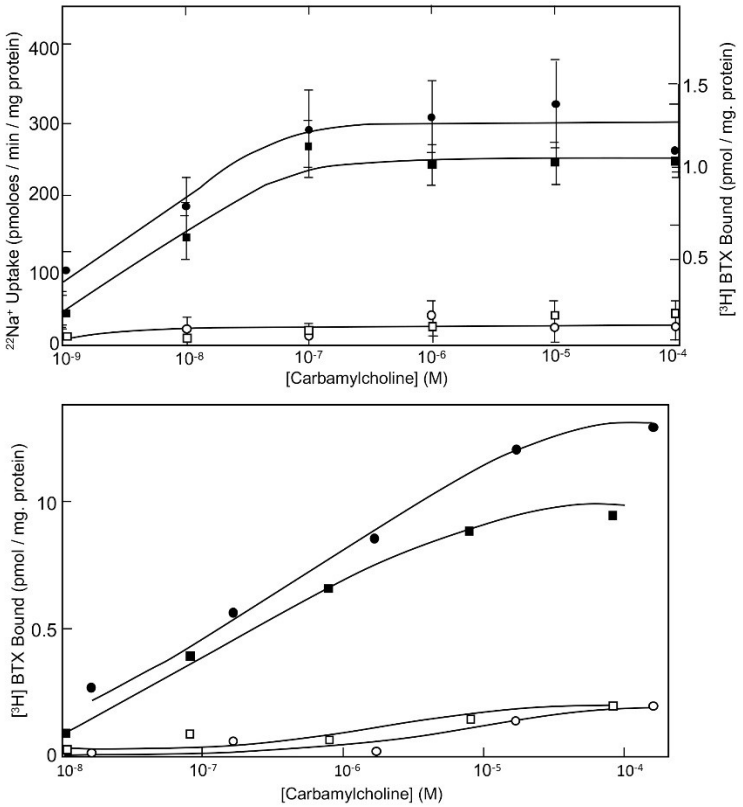


Figure 2

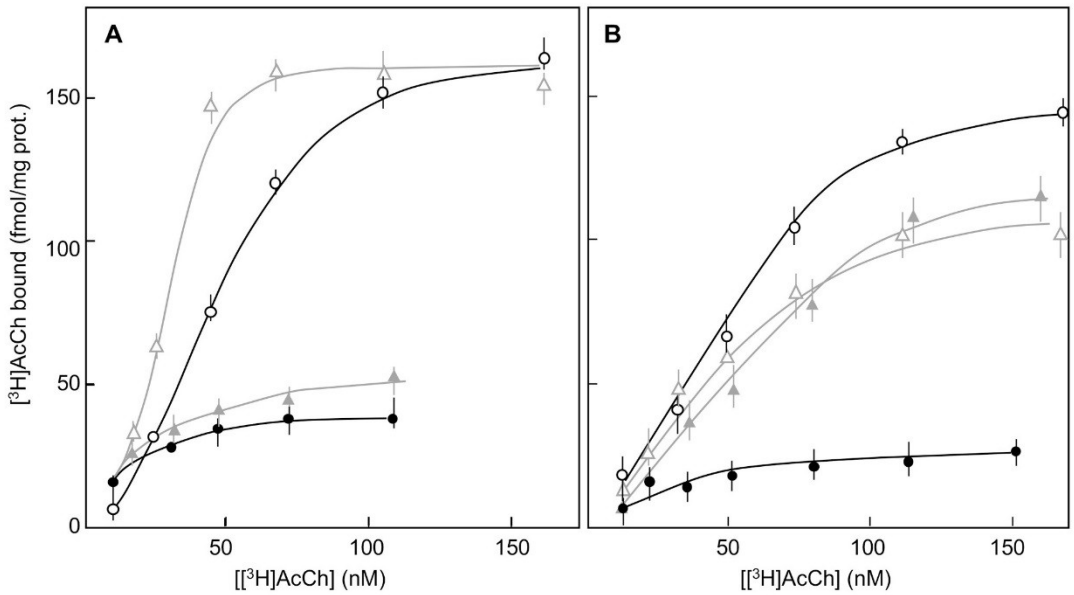
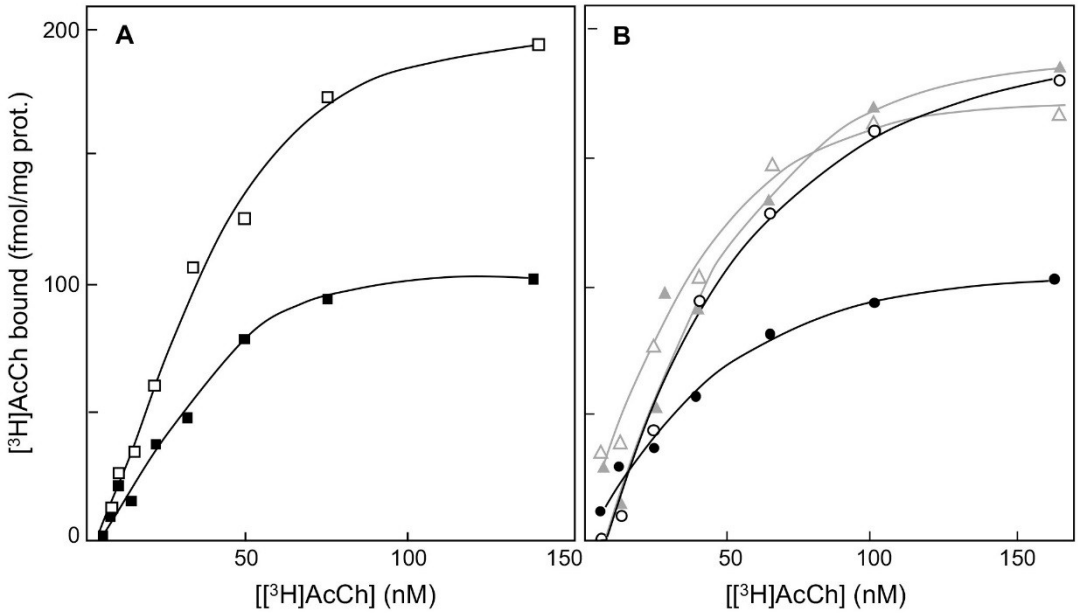


Figure 3


A
B
200
100
0
50
100
150
[³H]AcCh bound (fmol/mg prot.)
[[³H]AcCh] (nM)
[[³H]AcCh] (nM)